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RHINOVIRUSES AND RESPIRATORY DISEASE[1]

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INTRODUCTION

The first isolations of rhinoviruses were reported in 1956 by investigators in two separate laboratories (36, 39) who had noted the growth in monkey kidney (MK) cells of filterable agents associated with mild respiratory disease. These viruses were designated GL2060 by Pelon et al. (36) and JH by Price (39). They had many similarities, but initially their antigenic composition did not appear to be identical (29). Serological evidence was found for widespread infection in adults, and a causal relationship with respiratory disease was suggested (39). Additional serological studies showed a wide prevalence of JH neutralizing antibody in several areas of the world, and confirmed earlier findings that such antibody was acquired between infancy and the late twenties (36, 51).

Although growth in primary human embryonic kidney (HEK) cells was soon reported, supplies of such cells were limited, and early studies were hampered by the insensitiveness and variability of the tissue culture systems used (29). By 1958, the use of roller drums, rather than stationary racks, and of second generation MK cells had added significantly to the sensitivity of isolation methods (19, 41, 51). It then was reported that two JH serotypes existed, JH_2 being identical to GL2060 (41), and JH_1 being somewhat different on the basis of studies with human sera. JH_1 virus was the strain sent to other investigators (W. H. Price, *personal communication*), and subsequent volunteer studies demonstrated cross-protection between GL2060 and JH_1 (23). Since that time, 2060-JH has been considered a single serotype. In 1960, isolation of two new serotypes (HGP and FEB) was reported. This was achieved by use of HEK cell cultures in roller tubes and by incorporating two new features: a temperature of 33 C instead of the customary 37 C, and a more acidic medium (53). These studies were done by Tyrrell et al. (18, 52, 53) at the Common Cold Research Unit at Salisbury, England, and the strains were soon referred to as "Salisbury viruses." HGP virus was found to grow in both MK cells and HEK cells, while FEB virus grew only in the latter, thus demonstrating a differential trophism of rhinoviruses for the first time. Henceforth, strains were given an "H" (human cells) or an "M" (monkey cells) designa-

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tion. These strains were also shown to be inactivated by a pH of 2.0, a most useful characteristic in differentiating them from the acid-stable enteroviruses (53).

A significant contribution to the ease of working with rhinoviruses was the introduction of the use of the WI (Wistar Institute) strains of diploid human embryonic lung cells (15) by Hamparian, Ketler, and Hilleman (10). These investigators suggested that the new viruses be called "coryzaviruses." By use of HEK or WI cells and the special conditions for maintenance of cultures, many strains were soon isolated from adults and children with respiratory illness in various populations (10, 14, 19, 54). Individuals without respiratory symptoms were studied simultaneously as control populations, and the causal relationship of the viruses with mild upper respiratory disease was confirmed (10, 14). The WI cell strains proved to have greater and more consistent sensitivity to rhinoviruses, facilitating work with H strains, and it was shown that H strains are more prevalent than M strains (10, 14). M strain viruses were adapted to growth in several human embryo cell strains and in a wide range of continuous cell lines (30, 46); H strain viruses also were adapted to continuous cell lines, but with difficulty in some instances (27, 46).

Having been shown to possess characteristics similar to the enteroviruses, GL2060 virus initially was classified as ECHO 28 (37, 38). As subsequent strains were identified, ECHO 28 and the new isolates were referred to by an assortment of names: muriviruses, respiroviruses (31), Salisbury agents (53), coryzaviruses (10), rhinoviruses (1), and ERC (ECHO-Rhino-Coryza) viruses (27). By 1962, it became clear that there existed multiple serotypes of these viruses, at least 30 more being reported in a relatively brief period (24, 27, 45). Hamparian et al. (10) gave the numbers 11 through 30 to their serotypes so that previously reported serotypes could be given priority in numbering. Cross-reactions between serotypes were noted, but in most instances were found to be uncommon. A code for tentative classification was proposed, based on the place of isolation, sequence, and year that the original specimen was collected (45), and the name rhinovirus was adopted for the group at the Eighth International Congress of Microbiology (21, 56). Rhinoviruses were classified as a subgroup of the newly proposed Picornavirus group, a group established to include also the enterovirus subgroups polio, Coxsackie, and ECHO (21). At the present time, no standard system for numbering rhinovirus types has been adopted, but comparison of isolates should soon make such designation possible.

With improved techniques, spurred by the convinction that true "common cold viruses" had at last been found, knowledge of rhinoviruses accumulated rapidly. It is the purpose of this paper to review what has been learned about the biological characteristics and epidemiological behavior of this important new group of viruses. To data from the literature are added observations made at the University of Virginia since 1959 in two population groups: university students, and employees in the Eastern Regional Office of State Farm Insurance Co.

GROWTH OF VIRUS

Isolation Techniques

Rhinovirus isolations have been made from nasal or throat swabs or both, and from nasal washings. The latter method of specimen collection has been reported as giving the highest yield (2, 4), but it has practical drawbacks in most study populations. Attempts at rhinovirus isolations from the conjunctiva and feces have been unsuccessful (4, 24, 45). In our studies, a nasal or pharyngeal swab was collected from students, and both were obtained from insurance company employees and placed in a single vial.

Isolation attempts from naturally occurring infections and from illnesses in volunteers have shown virus shedding to persist for at least 3 to 4 days (16, 23, 34). In our own experience, there has been no difference in isolation rates among illnesses of 1, 2, or 3 days' duration. Attempts at virus isolation in the convalescent period following natural infection have shown virtual absence of virus (6). The fact that virus recovery rates at that time are as low as those observed for asymptomatic controls suggests active neutralization of the infecting virus.

The concentration of virus in original specimens appears to be low (41). In our own experience with WI cells, approximately 30% of the original positive specimens tested contained only one infectious dose of virus per 0.4 ml of collecting broth. In volunteer studies, virus isolation was a less sensitive indication of infec-

tion than was serological conversion with one H strain, although the two methods proved equally sensitive with an M strain (33, 34).

Specimens have been collected in various salt solutions or broth containing added protein for virus stabilization. We have used Beef Heart Infusion broth with 1.0% bovine serum albumin. In our experience, freezing original specimens once prior to testing did not reduce the rate of virus isolation beyond that which could have occurred due to chance. Others have shown that the largest loss of infectivity occurred after one freeze-thaw cycle and 6 months of storage, and that additional freeze-thawing and storage caused further, but less significant, loss. M strains were reisolated significantly more often than H strains after one or two freeze-thaw cycles (2).

Tissue Culture

On initial isolation, rhinovirus cytopathic effect (CPE) usually appears promptly in WI cells. In one study (2), it uniformly occurred within 5 days. In our experience, one-third of the strains produced no discernible CPE until the second week of observation. For this reason and because isolation of additional viruses such as adenovirus may occur later, tubes are observed for a minimum of 2 weeks. One blind passage in WI cells was reported not to increase rhinovirus yield (10). Herpesvirus and adenovirus usually can be distinguished in WI cells by the character of their CPE (2); this is also true of respiratory syncytial virus. Freeze-thawing WI cells during the harvesting of rhinovirus did not increase the titer; after adaptation had been achieved, titers of 10^5 to 10^6 were uniformly reached (6).

Studies done in our laboratory on specimens collected from college students have shown the superior sensitivity for primary rhinovirus isolation of HEK over MK cells and in turn of WI over HEK cells (Table 1). These specimens had been thawed and refrozen at least four times and stored at −70 C for 2 to 3 years before being tested in WI cells. Our studies with specimens from an industrial population have shown that the two strains of WI cells (26 and 38) used are of equal sensitivity for rhinovirus isolation (Table 2).

The composition of the WI maintenance media used by various investigators has not varied markedly (2, 6, 27). The presence or absence of serum in the medium has not significantly affected the growth of some rhinovirus strains (25, 53). In the past, efforts have been made to adjust initial pH values to rigidly set limits [pH 7.2 to 7.4 (10), 7.4 (27), 7.0 to 7.3 (25), or 6.8 (6)] with sodium bicarbonate or tris(hydroxymethyl)aminomethane buffer. After more experience with WI cells, rigid control of initial pH has not appeared as critical (25); this has also been our experience. We have used a WI maintenance medium consisting of 49% Eagle's Minimal Essential Medium, 49% Medium 199, and 2% inactivated fetal calf serum without further adjustment of initial pH, which has ranged from 7.1 to 7.6. Prior to subsequent feeding, medium pH values have fallen to 6.6 to 6.7. The medium also contains antibiotics (penicillin, streptomycin, kanamycin, and amphotericin), and L-glutamine is added at the time of use.

TABLE 1. *Rhinovirus isolations from students with respiratory symptoms**

Cell type	No. tested	Isolations	
		No.	Per cent
Monkey kidney	263	5	1.9
Human embryo kidney	175	7	4.0
Human embryo lung fibroblast (WI-26 or 38)	281	33	11.7

* Same specimens tested in three different types of cells; specimens had been frozen and thawed repeatedly and stored for 2 or more years before passage in WI cells.

TABLE 2. *Comparison of rhinovirus isolations in two lines of human diploid cells*

WI cell strain	Illnesses sampled*	Positive	
		No.	Per cent
26	232	51	22
38	78	19	24

* Different acute respiratory illnesses.

With WI cells, incubation at 33 C instead of 37 C has not appeared critical after initial virus isolation (10, 25). No data are available regarding optimal incubation temperature for original isolation attempts, and this is still held at 33 C in our laboratory. Motion has also been cited as not being necessary with WI cell cultures (25). It has

been the experience of others (6), supported by our own experience, that this is not the case. It is our impression that motion is particularly important for the development of maximal H strain CPE.

In addition to HEK, other tissue culture cells used in working with rhinoviruses are primary monkey kidney cells and, more recently, primary baboon kidney cells. As noted, the latter are used to demonstrate the differential trophism of rhinoviruses, with baboon cell sensitivity falling between that of monkey and human cells (R. M. Chanock, *personal communication*). Sensitivity for rhinovirus growth is enhanced if MK cells are used in the second generation (41). Because superior rhinovirus yield can be attained with KB cells after virus adaptation (25), this cell line has proved useful in antigen production. Wide variation in the sensitivity of primary cell strains (24) and continuous cells lines (46) has continued to be a problem; reports of variation of passages of human embryonic lung strains may be anticipated, but these diploid cells are far superior to other cells currently available.

PROPERTIES OF VIRUSES

Physical and Chemical Characteristics

Rhinovirus particle diameter has been studied by ultrafiltration, centrifugation, and electron microscopy; estimates of size have ranged from approximately 15 to 30 mμ (7, 10, 25, 41). One strain has been shown to have a definite substructure (27). A hydrated density of about 1.3 has been reported (7, 55). Infectious ribonucleic acid (RNA) has been extracted from one H strain (27), and many H and M strains have not been inhibited by 5-fluorodeoxyuridine, indicating RNA composition (6, 27).

The range of thermal stability has been evaluated for several strains. H and M strains can be stored at −70 C indefinitely, and M strains have been shown to remain stable for weeks to months at 4 C (6, 30). Three M strains have been shown to be rapidly inactivated ($<$ 30 min) at 56 C, but to be relatively stable at 50 C (6, 37, 53), as are H strains (12). ECHO and Coxsackie viruses are relatively labile at 50 C, a characteristic which proves useful in rhinovirus differentiation. Tests of a few rhinovirus strains for stabilization by 1 M Mg^{++} to 50 C for 60 min showed that stabilization occurred when sufficiently heat-sensitive strains were tested (7, 12). M strains have been tested for inactivation at 37 C, and the half-life of one strain was found to be 6.8 hr (6, 47).

The complete range of pH stability for these agents has not been reported in a sensitive tissue-culture system. Early reports suggested that stability decreased in alkaline solution (37). It is now well established that rhinoviruses are acid-labile at a pH of 3 to 5. All H and M strains tested to date have shown this characteristic (2, 6, 7, 27). Two satisfactory methods of testing for acid lability have been reported (55). The method used in our laboratory is as follows. Undiluted virus is mixed with 9 volumes of Hanks' balanced salt solution (BSS). One portion is then mixed with 2 volumes of glycine HCl buffer (pH 2.8) and another portion is mixed with the same volume of Hanks' BSS. The mixtures are held at room temperature for 3 to 4 hr, after which 0.1 volume of 4.4% sodium bicarbonate is added to the virus buffer mixture, and the mixtures are inoculated into WI tubes to determine infectivity. Acid lability is considered to exist if no CPE occurs in the tubes infected with the acid-exposed virus. Occasionally, high titered rhinoviruses will show false acid resistance with this and other methods (2). Therefore, resistant isolates are retested with the use of 100 to 300 $TCID_{50}$ in the portion mixed with glycine HCl buffer.

Ether stability, a general property of all Picornaviruses, has been shown to be a universal characteristic of H and M strains (6, 25, 27, 41, 52). Prototype strains have been tested in our laboratory with an alternate chloroform method (8); as expected, all were chloroform-stable. H and M strains have also shown resistance to fluorocarbon (10, 53).

Tests of Picornaviruses with 2-(hydroxybenzyl)-benzimidazole (HBB) and guanidine hydrochloride have shown selective inhibition (44). Rhinoviruses were HBB-insusceptible, a finding which has been confirmed for some H and M strains by other workers (2, 6). However, evidence has been presented that selective HBB inhibition of Picornaviruses appears to be too variable for use as a routine diagnostic tool for rhinovirus separation (11). Rhinovirus characteristics are summarized in Table 3.

Following a scheme of determining nucleic acid content, ether or chloroform sensitivity, and acid lability, an unknown isolate can be placed in its proper group (11). For tentative identification of a rhinovirus, we have followed the

simplified method shown in Fig. 1. Identification then proceeds with type-specific antisera in a neutralization test. The appearance of characteristic CPE, e.g., suggesting herpes simplex or respiratory syncytial viruses, permits immediate testing with specific antisera. Separation by H and M strains has been used in the past in our laboratory to limit the serotype possibilities of an unknown rhinovirus. This is not always reliable, and is helpful only if positive identification is achieved.

TABLE 3. *Rhinovirus characteristics*

Determination	Result
Biological characteristics	
Primary isolation*	Human embryonic lung
Cytopathic effect	Similar to other picornaviruses
Growth	Enhanced by motion, decreased temperature (33 C), and near neutral pH
Antigen	Type-specific
Complement-fixing antigen	Unsatisfactory
Hemagglutinin	None
Growth in laboratory animals	None
Changes in embryonated eggs	None
Physical and chemical characteristics	
Size	15 to 30 mμ in diameter
Shape	?Rhombic triacontahedron
Density	1.3 (hydrated)
Nucleic acid	RNA
Heat	Inactivated at 56 C, relatively stable at 50 C
Acid (pH 3 to 5)	Labile
Ether and chloroform	Resistant
Fluorocarbon	Resistant
Hydroxybenzyl-benzimidazole	Resistant
Guanidine HCl	Variable sensitivity

* H strains grow in human cells only; M strains grow in monkey and human cells. Rhinoviruses are adaptable to some human continuous cells.

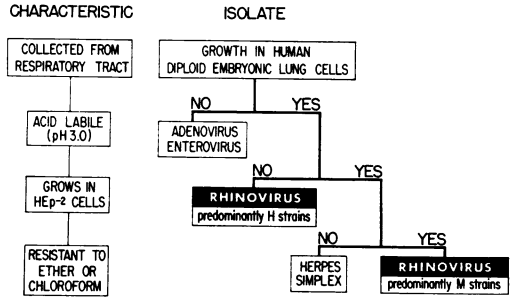

FIG. 1. *Schema for preliminary identification of rhinoviruses.*

Biological Properties

High-titer animal antisera production has been a major problem in rhinovirus investigation. This is especially true for the H strains (50). Guinea pigs have proven to be the most satisfactory small animals for antisera production (20), with the use of adjuvant being desirable to achieve higher titers (27). Recently, as part of the program of the Board for Vaccine Development of the National Institute of Allergy and Infectious Diseases, calf antisera have been produced with excellent titers and in quantities never before possible. Rhinoviruses as antigens in man will be discussed later.

Various proposed rhinovirus serotypes have been tested against antisera of a number of enteroviruses, including polio 1–3, Coxsackie A 1–19, 21 (Coe), Coxsackie B 1–5, ECHO 1–25, and Pett (6, 10, 25, 31, 36, 45, 53). With one exception, there has been no evidence of antigenic similarity. In one report, there was a possible slight relationship between rhinovirus B632 and Coxsackie A7 (45).

Attempts to demonstrate hemagglutinins by use of human, fowl, monkey, and guinea pig erythrocytes have failed (10, 36, 41). Likewise,

attempts to produce satisfactory complement-fixing antigens have been unsuccessful (6, 10, 25, 36, 41, 47).

M and H strain rhinoviruses have not grown or produced disease in mice, hamsters, guinea pigs, ferrets, or rabbits, or produced pathological changes in embryonated eggs (10, 25, 39).

PRESENT STATUS OF SEROTYPES

The methodology of rhinovirus neutralization tests is still undergoing evaluation. Increasing concentrations of antiserum have been shown to give greater rates of virus inactivation; varying virus inoculum has produced the same proportion of virus survival whether concentrated or dilute virus was used (47). A microplaque reduction method for measuring rhinovirus concentration (35) was developed, but has been abandoned in favor of the slightly less sensitive but simpler end-point method (50). The use of small doses of virus (10 $TCID_{50}$) has been found to be important in the end-point method when measuring rhinovirus-neutralizing antibody in humans. The duration and temperature of incubation of the virus-antiserum mixture has been felt by some to be important in controlling the results of neutralization tests, and perhaps may explain the differences in frequency of occurrence of distinct rhinovirus serotypes reported (31). As noted earlier, incubation of virus-serum mixtures at 37 C may cause some nonantibody inactivation of virus. Neutralization tests are done in our laboratory with a virus-serum incubation period of 2 to 3 hr at room temperature. When controls are positive, end points are read as the highest initial dilution of serum which caused 50% or greater suppression of cytopathology. Following the practice of Schieble and Lennette (*personal communication*), antisera currently are combined in intersecting pools in a manner similar to that used for enteroviruses.

At present, the total number of rhinovirus serotypes is unknown, and serological comparisons of the existing proposed serotypes is incomplete or not reported. To date, 37 proposed serotypes have been reported: 9 M strains and 28 H strains (Table 4). Recent unpublished data of Schieble and Lennette suggest that Chicago 179E(M) is similar to coryzavirus 30(M), and that NIH 353(H) and coryzavirus 23(H) are similar serotypes. If confirmed, these identities would reduce the number of serotypes, but many new isolates not yet reported in the literature are sure to boost the number toward 40 again.

We have isolated from university students one M strain and five H strains which have not been neutralized by all of the antisera available to us at this time. A summary of the status of serotypes isolated from this population is given in Table 5. Complete typing of these and the much greater number of isolates from an industrial population awaits production of additional antisera.

TABLE 4. *Proposed rhinovirus serotypes*

Strain	Reference	Serotype
M	36, 39	2060-JH
	53	Salisbury/1/57 (HGP)
	19	Shef/30/60
	31	K/779/59
	45	B632
	47	Coryzavirus 30
	6	Chicago 106F, 140F, 179E
H	53	Salisbury/1/58 (FEB)
	19	Shef/16/60, Shef No. Shef Thomp.
	42	Coryzavirus 11-29
	24	NIH 353, 1059, 1734, 33343, 11757

TABLE 5. *Serotypes of rhinoviruses isolated from university students with respiratory disease*

Strain	Serotype	No.
M	2060-JH	5
	Salisbury/1/57 (HGP)	3
	Chicago 106F	2
	Chicago 140F	2
	Chv/1/60	1
	Unidentified*	1
H	Salisbury/1/58 (FEB)	1
	Shef/16/60	1
	NIH 1734	2
	Chv/1/59	1
	Chv/2/59	3
	Chv/3/59	1
	Chv/7/59	1
	Chv/5/60	3
	Unidentified	6

* Versus antisera for types listed above in addition to Chicago 127-1, Salis Thompson, Salis B632, Chicago 106-F, Chicago 140-F, and Chicago 179 E.

EPIDEMIOLOGY

Prevalence

Except for one report of a rhinovirus isolation from calves (3), isolations so far have been exclusively from humans. Rhinovirus distribution has been shown to be world-wide by serological studies (49). Surveys for antibodies to several different M serotypes—2060-JH (36, 41, 49, 51), HGP and B632 (43, 49)—and several H serotypes—NIH 353, 1059, 1734, 11757, and 33343 (25)—show a prevalence of neutralizing antibodies in adults of approximately 50 to 70%. Studies with 2060-JH have shown no significant differences in prevalence on the basis of sex, race, and socioeconomic group (41).

Prevalence of neutralizing antibodies in infants and young children is in general infrequent or much less frequent than in adults (0 to 30%). Antibodies to all types tested so far begin appearing in older children and adolescents, and reach adult prevalence levels in the mid-twenties. When infected, children appear to respond with antibody titers which equal those occurring in adults. Consequently, lack of antibody in young children is probably the result of fewer rhinovirus infections (49, 50). Since rhinoviruses appear to spread relatively poorly (26, 34, 41), the lack of infection in young children is presumably due to less chance for infection because of limited cumulative contact possibilities. Antibody response following M strain infections appears with greater regularity than with H strain infections (50), a difference which affects the results of serological surveys of rhinovirus antibody prevalence.

In isolation studies, H strains predominate in spite of the apparent greater stability and ease of isolation of M strains. Reports show H strains to comprise 52 to 100% (2, 10, 14) of all rhinovirus strains isolated from adults. Our own data support this; 58% of the student isolates and approximately 90% of the industrial isolates are H strains. In children H strain isolates also have predominated with reported frequencies of 63% (10) and 93% (2). One study (2) has reported a seasonal variation in incidence of M strains with a peak of occurrence in the winter and spring; H strains occurred predominantly in the fall. The latter finding has received support in another report (42), and nearly all of our industrial M strain isolates were recovered in March and December.

Association with Disease

Rhinoviruses have been established as a cause of acute upper respriatory disease in adults and children (2, 12, 42). Rhinovirus isolation rates from adults with upper respiratory illness and well controls are shown in Table 6. Varying techniques of specimen collection and testing were used. Some specimens were tested fresh and some after freezing and storage. Table 7 details our isolation results in an industrial population of young adults in Charlottesville over a 12-month period in 1963–64. Approximately 33% of reported respiratory illnesses were tested; isolation rates from illnesses were highest in the spring and fall; the majority of isolations from controls were clustered in late spring. A change in the material used for specimen collection swabs may have been responsible for a fall in virus isolation rates during November, December, and the first half of January. With a return to cotton swabs, rates approached the earlier level. Rhinoviruses were isolated from 19.4% of those with acute respiratory symptoms and from 2.1% of those who had had no such symptoms for 2 weeks. Within the range from 18 to 35 years, virus recovery was not influenced by age, sex, or cigarette smoking.

Rhinovirus isolation attempts from military recruits with pneumonia have been unsuccessful (2). In the same study, rhinovirus infections were associated with febrile upper respiratory illness in recruits. However, because of the possibility of concomitant infection with other agents, this relationship has not been established. Other series, including our own, have shown only a small percentage of rhinovirus positive infections in civilian adults to be accompanied by temperature elevations.

Reported rhinovirus isolation rates from children and adolescents with upper respiratory illnesses and from well controls are shown in Table 8. Children and adults seem to be affected by the same serotypes as determined by serological studies and by virus isolations (42). The association of rhinoviruses with febrile upper respiratory illnesses in children has been reported (2), and some children with rhinovirus infections have had clinical features of lower respiratory tract involvement, i.e., bronchitis, croup, and bronchopneumonia (2, 17). However, a causal relationship with naturally occurring lower respiratory tract disease has not been definitely established

TABLE 6. *Frequency of rhinovirus isolations from adults*

Authors	Cells used	Population sampled	Respiratory illness			No illness		
			No. tested	Positive		No. tested	Positive	
				No.	Per cent		No.	Per cent
Hobson and Schild (19)	MK, HEK	Not stated	25	8	32	8	0	0
Hamre and Procknow (14)	MK, HEK	Medical students	199	39	20	456	2	0.4
Tyrrel and Bynoe (54)	MK, HEK	Lab staff	64	14	22	—	—	—
Reilly et al. (42)	WI-26	Industrial	140	20	14	48	1	2
Bloom et al. (2)	MK, WI-26	Military recruits	643	115	18	732	40	5
Bloom et al. (2)	MK, WI-26	Force troops	514	35	7	453	7	2
Higgins et al. (16)	MK, HEK	General practice	239	16	7	—	—	—
Authors' series	MK, HEK, WI-26	University students	286	33	12	—	—	—
Authors' series	WI-26 & 38	Industrial	433	84	19	810	17	2
Total			2,543	364	14.3	2,507	67	2.7

TABLE 7. *Isolation of rhinoviruses from employees with and without respiratory symptoms*

Month (1963–64)	Illness			No illness		
	No. tested	Positive		No. tested	Positive	
		No.	Per cent		No.	Per cent
March	60	10	17.7	95	1	1.1
April	55	13	23.6	93	3	3.2
May	26	8	30.8	65	4	6.2
June	23	5	21.7	60	5	8.3
July	16	2	12.5	66	0	0.0
Aug.	14	2	14.3	66	1	1.5
Sept.	55	18	32.7	60	0	0.0
Oct.	42	13	31.0	69	0	0.0
Nov.	17	0	0.0	53	0	0.0
Dec.	51	4	7.8	60	0	0.0
Jan.	36	3	8.3	66	3	4.5
Feb.	38	6	15.8	57	0	0.0
Total	433	83	19.5	810	17	2.1

at present because of the possibility of dual infections.

There is no definite evidence that these agents are associated with gastrointestinal illness. Volunteers given rhinovirus positive secretions from individuals with concurrent respiratory and gastrointestinal symptoms have failed to develop gastrointestinal symptoms (26).

Evidence for asymptomatic infection has been provided not only by studies showing rhinovirus isolations from asymptomatic individuals but also by serological studies of natural and experimental infections. The frequency of occurrence of asymptomatic infections determined by serological means has been reported to be from 9 to 27% (4, 23, 40). Evidence from natural infections (26, 41) and volunteer experiments (34) shows that secondary attack rates are low, and suggests that these viruses spread relatively poorly.

Clustering of infections due to a single serotype has been observed, (33, 39), suggesting that focal epidemics occur. It is too early to assess the relative importance of the various serotypes as a cause of respiratory illness, except for possibly 2060-JH. Several series using serological or isolation data have shown that 2060-JH was associated with 2 to 15% of respiratory illnesses (2, 6, 33, 41), and accounted for 9 to 21% of all rhinovirus isolates. In our student population, 2060-JH was isolated from 2% (5 of 286) of the illnesses, and accounted for 15% of the rhinoviruses isolated. It was associated with a fourfold or greater rise in neutralizing antibody titer in 5% (10 of 215) of paired sera.

CLINICAL CHARACTERISTICS OF RHINOVIRUS ILLNESS

Efforts to separate mild respiratory illnesses etiologically on the basis of anatomically oriented syndromes have met with little success in the past. Unrelated respiratory viruses can cause similar clinical manifestations; variable clinical

pictures can result from infections with the same virus. This difficulty is particularly apparent when trying to assign an etiology to an individual case. However, with specific etiological agents now available in greater number, it appears that some large clinical categories may be emerging.

Interpretation of various studies is influenced by the criteria used in selection of cases for study. In some, these criteria are not stated, and in all they vary. Our simple criteria for a respiratory illness in insurance company employees were at least two symptoms on 1 day or one symptom on 2 days or more, at least one of the symptoms being of respiratory origin. Illnesses of over 3 days' duration were not tested. Variables such as age, sex, and cigarette smoking need further evaluation for the part they may play in the clinical response to respiratory virus infection.

Volunteer studies with JH and 2060 strains in adults have shown an incubation period of less than 24 hr, with a peak frequency and severity of symptoms of 48 to 120 hr. Duration of symptoms has been approximately 1 week (23). In naturally occurring rhinovirus illnesses in adults, symptom duration has been 4 to 24 days with mean durations of 7 to 10 days (14). Duration of symptoms in rhinovirus-positive illnesses in the insurance company employees ranged from 1 to 23 days, with a mean of 7.4 days, and a median of 6.4 days with a quartile deviation of ± 3.2 days.

Studies of the signs and symptoms associated with 110 JH infections showed no significant differences on the basis of race or sex. Age differences did not appear marked, but significance was not assessed (41). A comparison of illnesses in adults and children with H strain infections showed more frequent occurence of fever and lower respiratory tract manifestations in the children (42). Another study found that H and M strain rhinovirus illnesses were no more severe in infants and young children than in older children (2). As noted earlier, the question of occurrence of lower respiratory tract disease in children due to rhinovirus infections remains unsettled.

Two cross-sectional studies of upper respiratory illness in older children and adults have compared rhinovirus positive and negative illnesses. In one instance, patients infected with rhinoviruses had more hoarseness and less headache, cough, pharyngeal involvement, and diarrhea than rhinovirus-negative patients (26). The significance of these differences was not reported, however. In the other study (9), rhinovirus-positive individuals had significantly greater total symptom scores, and more sneezing and chest pain, than did virus negative individuals. Also, rhinovirus-positive illnesses had significantly less sore throat, cough, chills, myalgia, anorexia, and fever, and significantly more sneezing and chest pain than adenovirus-positive illnesses. When compared with Coe virus (Coxsackie A21) infections, rhinoviruses were associated with significantly more chest pain, cough, and vomiting and significantly less conjunctival imvolvement. Some of these observations were made during different time

TABLE 8. *Frequency of rhinovirus isolations from young children and from adolescents and older children*

Age group	Authors	Cells used	Population sampled	Respiratory illness			No illness		
				No. tested	Positive		No. tested	Positive	
					No.	Per cent		No.	Per cent
Young children	Reilly et al. (42)	WI-26	Outpatients	263	15	6	81	1	1
	Bloom et al. (2)	MK, WI-26	Military dependents	741	29	4	825	14	2
	Higgins et al. (16)	MK, HEK	General practice	96	2	2	—	—	—
	Total			1,100	46	4.1	906	15	1.6
Adolescents and older children	Kendall et al. (26)	HEK	Boarding students	59	18	30	—	—	—
	Higgins et al. (16)	MK, HEK	General practice	93	5	5	—	—	—
	Total			152	23	15.1	—	—	—

Volunteer studies with JH and 2060 strains in adults have shown an incubation period of less than 24 hr, with a peak frequency and severity of symptoms of 48 to 120 hr. Duration of symptoms has been approximately 1 week (23). In naturally occurring rhinovirus illnesses in adults, symptom duration has been 4 to 24 days with mean durations of 7 to 10 days (14). Duration of symptoms in rhinovirus-positive illnesses in the insurance company employees ranged from 1 to 23 days, with a mean of 7.4 days, and a median of 6.4 days with a quartile deviation of ± 3.2 days.

intervals and may reflect the occurrence and symptomatology of undetected agents. This seems especially likely with regard to gastrointestinal symptoms, which have not been prominent in other rhinovirus series.

Our own experience with the insurance population is illustrated in Fig. 2. Rhinovirus-positive illnesses were found to have significantly more rhinorrhea, cough, and observed nasal discharge. A comparison of clinical findings in the student population, with fewer virus-positive illnesses

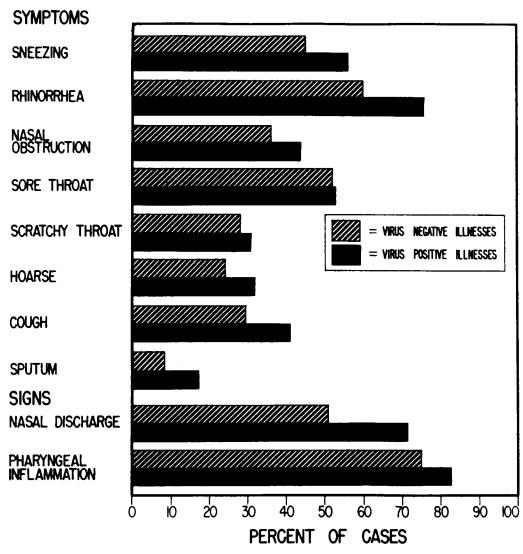

FIG. 2. *Clinical manifestations in insurance company employees of respiratory illnesses from which rhinoviruses were isolated compared with those of illnesses from which rhinoviruses were not isolated.*

included, revealed similar trends. An unresolved question is whether or not the mere presence of profuse nasal discharge facilitates the recovery of virus.

The mean temperature of 82 individuals in the industrial population with rhinovirus respiratory illness of 3 days' duration or less was 97.9 F, with a range of 96 to 99.8 F. In the university students, some of the rhinovirus positive illnesses, were accompanied by leukocyte counts of 10,000 per cm^2 or above, supporting other evidence that elevation of leukocyte counts can occur in rhinovirus illnesses in adults (42).

It should be noted that our criteria for the presence of respiratory illness in the insurance workers were more liberal than those reported by Kendall (26), who made rhinorrhea a prerequisite for inclusion in his study. Also, the illnesses studied in the industrial population were in general milder, with less systemic and lower respiratory tract symptoms, than those studied at our Student Health Service or those reported by Forsyth et al. (9). We are in agreement with the experience of Jackson et al. (22) that physical signs are of minimal help in establishing the diagnosis of a cold.

Longitudinal comparisons of rhinovirus-positive and -negative illnesses have been reported to show significantly greater total symptomatology in the rhinovirus-positive group (9). Data from our insurance company study reveal a mean duration of symptoms of 7.4 days for rhinovirus-positive illnesses and of 6.2 for rhinovirus-negative illnesses. This difference is not significant. A longitudinal comparison of total symptoms scores in the two groups reveals significantly more symptoms occurring in the virus-positive group, but no significant difference in occurrence of individual symptoms.

Evidence from some volunteer studies has suggested that the average clinical picture produced by some strains of rhinovirus differs from that produced by others (54). Other volunteer studies have failed to demonstrate induction of consistent patterns of illness with a particular virus (49).

ANTIBODY RESPONSES IN MAN AND USE OF VACCINES

Homologous Neutralizing Antibody Responses and Protection Against Infection

Studies of naturally occurring infections and those induced in volunteers have shown that M strain rhinovirus infections produce sufficient antigenic stimulus to be associated frequently with significant homologous neutralizing antibody responses (13, 16, 23, 39, 47, 53). Individuals infected with H strains have not shown such consistent increases in antibody titer, nor have the levels reached been as great (10, 25). M strain infections are associated with almost uniform (90%) appearance of neutralizing antibody, while present methods are able to measure such response much less frequently (37%) following H strain infections (50). Our limited data support this finding (Table 9). Overall, 70% of students with rhinovirus illnesses showed a fourfold or greater rise in titer of neutralizing antibody; 92% responded to M strains, 53% to H strains. Anti-

body responses of children, as previously noted, do not appear to be less than those of adults.

The height and duration of antibody response has been studied, but information is incomplete for many serotypes at this early stage. Twelve of fifteen (80%) patients showing significant antibody rises to M strain infections developed neutralizing antibody levels of 1:128 or greater. One of seven (14%) patients with significant antibody rises to Hstrain infections achieved a level of 1:128 or greater (50). In another report, 7 of 24 (29%) H strain infections showing significant rises induced convalescent antibody levels of 1:64 or greater (27). In our student cases, 7 of 11 (64%) significant M strain responses attained levels of 1:64 or greater, while only one of eight (13%) significant H strain rises reached this level.

TABLE 9. *Homologous rhinovirus neutralizing antibody responses in university students*

Antibody increase	M Strains		H Strains	
	No.	Per cent	No.	Per cent
None	1	8	5	33
< Fourfold	0	0	2	13
Fourfold or >	11	92	8	53
Total tested	12	100	15	100

High levels of neutralizing antibody to M strain rhinoviruses have been shown to persist for months and years (6, 26, 41, 48). Existing data for H strains are scant, but suggest that antibody levels fall at a more rapid rate (48). Evidence has also been cited to show what appear to be occasional heterotypic neutralizing antibody responses in man, suggesting that rhinovirus types may share minor antigenic relationships not disclosed by current neutralization tests (6, 49). Controversy exists as to the frequency with which this occurs (31), but recent experience suggests that there is little crossing. Further standardization of specific sera and test methods is desirable.

Repeated experiments have sought to relate the presence of rhinovirus neutralizing antibody to protection against infection and illness. Volunteer studies have shown protection on rechallenge with the same M or H strain rhinovirus following the initial experimental infection (23, 24). Early volunteer studies investigating naturally occurring neutralizing antibody and resistance to infection or illness were contradictory or equivocal (23, 41, 51). More recent volunteer studies with both H and M strains indicate that pre-existing neutralizing antibodies are protective (4, 34). Naturaliy acquired H strain neutralizing antibody titers of 1:128 or greater were associated with absence of respiratory illness on virus challenge in 86% (18 of 21) of volunteers. Naturally acquired and vaccine-induced M strain neutralizing antibody titers of 1:32 or greater were associated with absence of respiratory illness on challenge in 93% (14 of 15) of volunteers. Prechallenge antibody levels of 1:4 or less were associated with illness rates of 81% (13 of 16) and 56% (5 of 9) for H and M strains, respectively (34). When illness did occur, however, the severity was not related to pre-existing antibody levels (4, 34).

Substances in the γ-globulin fraction of nasal secretions which inactivate rhinoviruses have also been demonstrated (41). Some such substances, found to be present in infectious secretions containing unknown agents, have been shown to be antibody (28). Levels of neutralizing antibody in respiratory secretions are stated normally to be well below those found in serum (1:250) (34). However, with the inflammation which occurs with respiratory infections, or even nonspecific irritants, the level of neutralizing antibody in nasal secretions increases; it has been found at one-tenth of that of serum in JH infections (41). Increased antibody begins to appear prior to clinical symptoms and provides evidence for understanding the occurrence of some asymptomatic rhinovirus infections (28), and perhaps is the mechanism which eliminates virus from the nose.

Rhinovirus Vaccines

Several investigators have demonstrated that formalin-inactivated 2060-JH vaccines are immunogenic in man. Stimulation of significant neutralizing antibody levels and protection against natural or experimental illness have been demonstrated (20, 32, 34, 40). There is one report of parenteral administration of live M strain rhinovirus to volunteers; high antibody levels were obtained and respiratory symptoms did not develop (5). Because of oncogenesis in animals associated with the parenteral administration of some live respiratory viruses, this approach has not been actively pursued. More promise appears to be found in the observation that volunteers fed live HGP rhinovirus developed significant rises in

neutralizing antibody without concurrent respiratory symptoms (49).

In an early field trial using formalin-inactivated 2060-JH vaccine, the attack rates for this virus during an outbreak were approximately eight times lower in vaccinated children than in controls (39). Large field trials with respiratory vaccines containing rhinoviruses have been undertaken by only one group of investigators (32). Multivalent vaccines containing 2060-JH virus and various combinations of influenza viruses, parainfluenza viruses, adenoviruses, and respiratory syncytial virus were used. Because these vaccines contained only one rhinovirus serotype and because 2060-JH virus was not shown to be producing illness at the time of the trials, the 13 to 33% "relative reduction" in rate of respiratory illness reported is difficult to interpret. At present, rhinovirus vaccine development is in its early stages. Because of the large number of distinct serotypes involved, and with many H strains showing poor antigenicity, it appears that numerous difficulties lie ahead. With continued improvement in the techniques of vaccine development and production, such as concentration of purified antigens and use of adjuvants, perhaps these difficulties will not be insurmountable.

COMMENTS

Rapid advances in the techniques of rhinovirus research have resulted in the opening of major areas for study of acute respiratory illness. Rhinovirus respiratory disease appears to be somewhat unique in the field of infectious disease in that large numbers of distinct etiological agents appear to be constantly present in the population causing similar clinical manifestations. It is possible that successive focal epidemics due to different types, but clinically indistinguishable, maintain a rather constant level of respiratory illness. An analogous situation exists with minor nonbacterial infectious gastroenteritis, although in this case the number of distinct etiological agents is unknown.

At the present time, a conservative estimate would relate rhinovirus etiology to approximately one-fifth of all minor respiratory symptoms occurring in adults. A portion of the remainder of symptoms can be accounted for by other known viruses and bacteria or by physical, chemical, allergic, or psychosomatic causes. This leaves an unknown, but probably sizeable, percentage due to undiagnosed rhinovirus infection or to infection with currently undetected agents.

Acute respiratory illness in infants and young children has not been associated with rhinovirus infection to any great extent, although older children and adolescents apparently are affected to a degree similar to adults. The low rhinovirus attack rates in preschool and grammar school children are in marked contrast to those for respiratory syncytial and parainfluenza viruses in these age groups. This is all the more perplexing in light of the knowledge that respiratory disease rates in adults are increased by exposure to children in this age range. The reasons for the different epidemiological behavior of these viruses is unknown, and is an area requiring further study.

Other needs relating to rhinovirus investigation at the present time are: a permanent system of numbering; better standardization of reagents, including typing sera; completion of serotype isolation; and definition of epidemiological behavior of various serotypes. Full description of the pathogenesis and characteristics of rhinovirus infection, an understanding of the basis for M and H strain differences, and a more complete knowledge of virus particle characteristics are among areas of study for the future. The numerous serotypes and expected difficulties in rhinovirus vaccine development also serve as another stimulus to the search for effective antiviral chemotherapy or chemoprophylaxis.

The search for "the common cold virus," overly optimistic in some ways, has been rewarded by the discovery of a unique and interesting group of viruses. Knowledge of rhinoviruses has greatly expanded the understanding of common respiratory disease and should eventually play a major part in its control.

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